Studies on the Sapogenins of Dioscorea tokoro Makino. II[1]. *The Structure of Tokorogenin*

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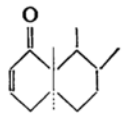
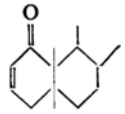
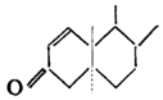
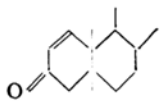
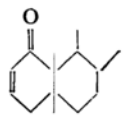
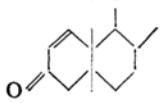
Although it was postulated that the three hydroxyl groups in tokorogenin are situated at positions 1, 2 and 3 of the steroid nucleus[2], their configuration and the nature of the A/B ring junction still remained unclarified. To make these points clear it was most desirable to establish a correlation of tokorogenin with a known sapogenin, which has been done as described in the present paper.

In the presence of a small amount of chlorosulfonic acid tokorogenin reacted with acetone and with cyclohexanone to give an isopropylidenetokorogenin (tokorogenin acetonide) and a cyclohexylidenetokorogenin, respectively. On treatment with pyridine and *p*-toluenesulfonyl chloride, the isopropylidenetokorogenin gave a tosylate, which on heating in 80% acetic acid solution furnished tokorogenin monotosylate. When the monotosylate was further treated with alkali in methanol, a crystalline, substance, soluble with difficulty, was obtained and analyzed as $C_{27}H_{42}O_4$. It was presumed to be an oxido-spirostanol,

1) This is a full paper of the previous communication (K. Morita, *Pharm. Bull.*, **5**, 494 (1957).), and constitutes Part IX of Nishikawa's paper entitled "Studies in Steroids". Part VIII: (K. Morita, This Bulletin, **32**, 476 (1959)).

2) Studies on the Sapogenins of *Dioscorea tokoro* Makino. I. (K. Morita, This Bulletin, **32**, 476 (1959).)

TABLE I

Chromophor	Substance	U. V. Spectra Max.	Log	Solvent	Ref. No
	5α-Cholest-2-en-1-one	224; 333	3.90; 1.75	C_2H_5OH	7)
	Methyl 1-keto-5β-eti-2-enate	225; 333	3.93; 1.96	C_2H_5OH	7), 8)
	Methyl 3-keto-5α-eti-1-enate	230; 323	4.07; 1.62	C_2H_5OH	7)
	Methyl 3-keto-5β-eti-1-enate	230; 323	4.12; 1.59	C_2H_5OH	9)
	25D-5β-Spirost-2-en-1-one (VII)	225; —	3.89; —	C_2H_5OH	*
	25D-5β-Spirost-1-en-3-one	232.5; —	3.99; —	C_2H_5OH	**

* This paper ** The subsequent report (Part III)

7) F. Sallman and Ch. Tamm, *Helv. Chim. Acta*, **39**, 1340 (1956).
8) W. Schlegel and Ch. Tamm, ibid., **40**, 160 (1957).
9) W. Schlegel, Ch. Tamm and T. Reichstein, ibid., **38**, 1013 (1955).

anhydrotokorogenin, from the reaction sequence. The anhydrotokorogenin gave a monoacetate and infrared absorption spectra exhibiting no characteristic band of the carbonyl group. It is, therefore, evident that the substance possesses a hydroxyl an oxido group. Oxidation of the anhydrotokorogenin with chromic anhydride-pyridine furnished a ketone, anhydrotokorogenone.

In the light of the structure of tokorogenin suggested in the previous paper[2] and the reaction sequence described above, the anhydrotokorogenone is either 2,3-oxido-spirostan-1-ol or 1,2-oxido-spirostan-3-ol. Since such an α,β-oxido-ketone has been known to undergo reduction with chromous chloride[3] giving the corresponding α,β-unsaturated ketone, the reaction was applied to the anhydrotokorogenone. As expected, anhydrotokorogenone gave an α,β-unsaturated ketone, which showed a specific U. V. absorption band at 225 mμ.

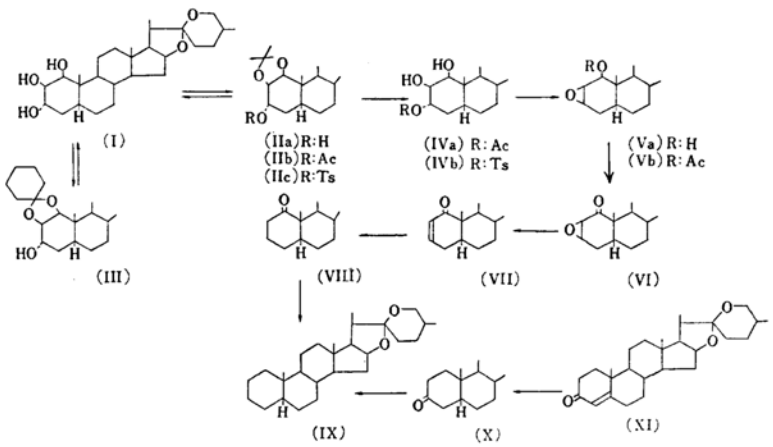
Catalytic hydrogenation of the α,β-unsaturated ketone furnished a saturated ketone, which showed marked depression of the melting point on admixture with smilagenone (X)[4]. Finally, the product from the Huang-Minlon reduction[5] of the saturated ketone proved to be identical with 25D-5β-spirostan (IX), prepared from smilagenone (X) by the known method[6]. The identity was established by the mixed melting point and the infrared absorption spectra.

The above reactions correlate tokorogenin with the known 25D-5β-spirostan (IX), and show that tokorogenin is an iso-sapogenin. Since the saturated ketone was not identical with smilagenone (X) as mentioned above, the structure 25D-5β-spirostan-1-one (VIII) has to be assigned to the saturated ketone from tokorogenin, and accordingly 25D-5β-spirost-2-en-1-one

3) W. Cole and P. L. Julian, *J. Org. Chem.*, **19**, 131 (1954).

4) The sample of smilagenone (X) was synthesized from diosgenin by Oppenauer oxidation followed by catalytic hydrogenation. C. Djerassi, R. Yashin and G. Rosenkranz, *J. Am. Chem. Soc.*, **74**, 422 (1952).
5) Huang-Minlon, ibid., **71**, 3301 (1949).
6) C. Djerassi and J. Fishman, ibid., **77**, 4291 (1955).

Chart 1



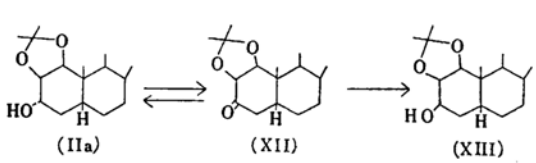
(VII) to the α,β-unsaturated ketone. These conclusions are further supported by the ultraviolet absorption spectrum of the α,β-unsaturated ketone (VII) which is in complete accord with those of known Δ^2-1-ketosteroids as shown in Table. I.

Thus, it is reasonable to assign the structure of $2\xi,3\xi$-oxido-25D-5β-spirostan-1-one to anhydrotokorogenone and that of $2\xi,3\xi$-oxido-25D-5β-spirostan-1ξ-ol to anhydrotokorogenin. Since the two hydroxyl groups in positions 1 and 2 in tokorogenin take part in the formation of the acetonide and therefore are *cis*-oriented, and the third in position 3 is *trans* to the other two, tokorogenin is either $1\alpha,2\alpha,3\beta$-triol or $1\beta,2\beta,3\alpha$-triol.

From the above arguments and on the basis of the assumption that the configuration of the hydroxyl group in position 3 of tokorogenin is α, all the reactions can be represented by the scheme shown in Chart 1.

Evidence for the α-configuration of the 3-hydroxyl group is given by the following experiment: Treatment of isopropylidenetokorogenin (IIa) with chromic anhydride-pyridine furnished isopropylidenetokorogenone (XII). Reduction of the ketone (XII) with lithium aluminum hydride gave a mixture of isopropylidenetokorogenin (IIa) and its C_3-epimer (XIII), which were separated by careful chromatography on Florisil, the latter being eluted first and then the former.

Chart 2



It has been generally accepted[8] that when a mixture of two epimeric steroidal alcohols is chromatographed, the one having an axial hydroxyl group moves more rapidly than the other. The above experiment, therefore, suggests that the 3-hydroxyl group in isopropylidenetokorogenin (IIa) is α-oriented (equatorial). In this way the structure of tokorogenin has been determined to be 25D-5β-spirostan-$1\beta,2\beta,3\alpha$-triol (I).

Experimental

Isopropylidenetokorogenin (Tokorogenin Acetonide) (IIa).—a) A suspension of 1 g. of tokorogenin (I) in 100 ml. of acetone containing 0.1 ml. of chlorosulfonic acid was vigorously stirred at room temperature for sixty minutes. When tokorogenin was dissolved and the acetonide was completely precipitated, the precipitates were collected, washed with ethanol and dried, m. p. 290~295°C. Yield, 1.0 g.

For analysis the substance was recrystallized from a large volume of hot methanol or dimethylformamide, m. p. 309~312°C.

Anal. Found: C, 73.50; H, 9.90. Calcd. for $C_{30}H_{48}O_5$: C, 73.73; H, 9.90%.

b) A solution of 100 mg. of tokorogenin in 100 ml. of acetone containing 50 mg. of *p*-toluenesulfonic acid was boiled under reflux for three hours and the solution was allowed to stand at room temperature overnight to separate fine needles of tokorogenin acetonide, m.p. 300~305°C, mixed m. p. with the above specimen, 305~309°C. When the acetonide was boiled in 80% acetic acid for several minutes and the solution was cooled, tokorogenin was precipitated in colorless needles melting at 273~275°C.

Cyclohexylidenetokorogenin (III).—Tokorogenin in cyclohexanone was treated with a few drops of chlorosulfonic acid under the conditions

described in the case of the acetonide. Recrystallization of the crude product from methanol furnished III melting at 288~290°C.

Anal. Found: C, 74.91; H, 10.04. Calcd. for $C_{33}H_{52}O_5$: C, 74.96; H, 9.91%.

When the cyclohexylidene compound (III) was boiled in ethanol containing a few drops of hydrochloric acid for one hour, tokorogenin was recovered.

Isopropylidenetokorogenin Acetate (IIb). — Isopropylidenetokorogenin (IIa) was acetylated by the usual means with pyridine and acetic anhydride to give the acetate (IIb), m. p. 207~208°C.

Anal. Found: C, 72.42; H, 9.70. Calcd. for $C_{32}H_{50}O_6$: C, 72.41; H, 9.50%.

Tokorogenin Monoacetate (IVa).—Isopropylidenetokorogenin acetate (IIb) was boiled in 80% acetic acid and the solution was cooled to separate colorless needles of tokorogenin monoacetate (IVa). The analytical sample recrystallized from methanol melted at 237~239°C.

Anal. Found: C, 70.75; H, 9.61. Calcd. for $C_{29}H_{46}O_6$: C, 70.98; H, 9.45%.

Isopropylidenetokorogenin Tosylate (IIc). —To a solution of 2.3 g. of isopropylidenetokorogenin (IIa) in 80 ml. of pyridine 5 g. of *p*-toluenesulfonyl chloride was added and the mixture was kept at 50°C for one hour and then at room temperature for two days. Then a large volume of water was added and the precipitate of the crude tosylate (IIc) was filtered, washed with water, and dried. Recrystallization from ether afforded colorless platelets melting at 198°C followed by immediate decomposition to a tar. Yield, 2.0 g.

Anal. Found: C, 68.94; H, 8.63. Calcd. for $C_{37}H_{54}O_7S$: C, 69.13; H, 8.47%.

Tokorogenin Monotosylate (IVb).—A suspension of 500 mg. of isopropylidenetokorogenin tosylate (IIc) in a small volume of 80% acetic acid was heated on a steam bath for twenty minutes till the solution became clear. The solution was then cooled to furnish colorless needles, m. p. 192°C (decomp.). Yield, 370 mg.

Anal. Found: C, 67.28; H, 7.96. Calcd. for $C_{34}H_{50}O_7S$: C, 67.75; H, 8.36%.

2β, 3β-Oxido-25D-5β-spirostan-1β-ol (Anhydrotokorogenin) (Va).—A methanol solution of 2.0 g. of potassium hydroxide was added to a solution of 1.0 g. of tokorogenin monotosylate (IVb) in 80 ml. of methanol and the solution was boiled under reflux for one hour and cooled. The hardly soluble crystalline solid was collected, washed with methanol, and dried, m. p. 237~239°C. Yield, 500 mg.

Anal. Found: C, 75.44; H, 9.88. Calcd. for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83%.

2β, 3β-Oxido-25D-5β-spirostan-1β-ol Acetate (Vb). — A portion of 2β,3β-oxido-25D-5β-spirostan-1β-ol (Va) was acetylated by the usual means with pyridine and acetic anhydride to furnish the acetate (Vb) as colorless prisms melting at 246~249°C.

Anal. Found: C, 73.51; H, 9.49. Calcd. for $C_{29}H_{44}O_5$: C, 73.69; H, 9.38%.

2β, 3β-Oxido-25D-5β-spirostan-1-one (Anhydrotokorogenone) (VI). — To a solution of 800 mg. of 2β,3β-oxido-25D-5β-spirostan-1β-ol (Va) in 50 ml. of pyridine was added with vigorous stirring 1.0 g. of chromic anhydride in small portions. The reaction mixture was gradually heated to 40°C and was kept at this temperature for three hours and then at room temperature for twenty-four hours. Water was added and the mixture was extracted with ether. The ethereal solution was thoroughly washed and dried, and evaporation afforded 700 mg. of needles, m. p. 238~239°C, I. R. in Nujol λ_{max} 5.86 μ (1-c=o).

Anal. Found: C, 75.35; H, 9.40. Calcd. for $C_{27}H_{40}O_4$: C, 75.66; H, 9.41%.

25D-5β-Spirost-2-en-1-one (VII).—To a suspension of 650 mg. of 2β,3β-oxido-25D-5β-spirostan-1-one (VI) in 5 ml. of acetic acid was added 20 ml. of a chromous chloride solution[10] at one time with vigorous stirring in nitrogen stream. The oxide (VI) dissolved immediately and soon 25D-5β-spirost-2-en-1-one (VII) was precipitated. Water was added and the mixture was extracted with ether. The ethereal solution was thoroughly washed and dried and evaporation furnished colorless needles, m. p. 222~223°C. Yield, 320 mg.; U. V. in EtOH λ_{max} 225 mμ (ε: 7690).

Anal. Found: C, 78.54; H, 9.51. Calcd. for $C_{27}H_{40}O_3$: C, 78.59; H, 9.77%.

25D-5β-Spirostan-1-one (VIII).—A solution of 300 mg. of 25D-5β-spirost-2-en-1-one (VII) in 50 ml. of methanol was subjected to catalytic hydrogenation over 1 g. of 5% palladium-charcoal catalyst. When 16 ml. of hydrogen was absorbed in five minutes the catalyst was removed and the filtrate was evaporated to give colorless prisms, m. p. 183~184°C. Yield, 220 mg.

Anal. Found: C, 78.17; H, 10.00. Calcd. for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21%.

25D-5β-Spirostan (IX). — a) A mixture of 150 mg. of 25D-5β-spirostan-1-one (VIII), 5 ml. of diethylene glycol and 0.2 ml. of 80% hydrazine hydrate was heated to 160~170°C till the solution became clear. Potassium hydroxide (300 mg.) was added to the solution and the temperature was gradually raised to 200°C and kept there for two hours. After cooling, water was added and the mixture was extracted with petroleum ether. Evaporation of the thoroughly washed and dried extract furnished a crystalline solid. Recrystallization from methanol containing ether afforded colorless platelets, m. p. 136~137°C. Yield, 98 mg.

Anal. Found: C, 80.62; H, 11.05. Calcd. for $C_{27}H_{44}O_2$: C, 80.94; H, 11.07%.

b) Smilagenone (X) was subjected to the same treatment as the above to give 25D-5β-spirostan (IX).

Comparison of the I. R.-spectra and mixed melting point determination with the two specimens showed that they were in complete accord in all respects.

25D-5β-Spirostan-1β, 2β-diol-3-one Acetonide (Tokorogenone Acetonide) (XII).—To a solution of 1 g. of tokorogenin acetonide (IIa) in

10) A solution of chromous chloride was prepared by the method of Cole and Julian[3].

150 ml. of pyridine was added 700 mg. of chromic anhydride in small portions with vigorous agitation. Temperature was kept at 45~50°C for forty hours, and stirring was continued throughout this time. After cooling, the mixture was diluted with a large volume of water and extracted with ether. The ether solution was thoroughly washed, dried and evaporated. Recrystallization of the residue from methanol yielded colorless needles melting at 225~228°C. Yield, 800 mg. The analytical sample recrystallized from the same solvent melted at 229~231°C. I. R. in Nujol, λ_{max} 5.80 μ (3-c=o).

Anal. Found: C, 73.98; H, 9.29. Calcd. for $C_{30}H_{46}O_5$: C, 74.03; H, 9.53%.

Lithium Aluminum Hydride Reduction of 25D-5β-Spirostan-1β, 2β-diol-3-one Acetonide (Tokorogenone Acetonide) (XII).—A solution of 300 mg. of $LiAlH_4$ in 50 ml. of anhydrous ether was added in small portions to a solution of 200 mg. of 25D-5β-spirostan-1β,2β-diol-3-one acetonide (XII) in 150 ml. of the same solvent. After being left to stand overnight at room temperature, the reaction mixture was poured into ice-water and extracted with methylene chloride. The organic layer was washed with water, dried, and evaporated to give a crystalline solid, which was re-dissolved in methylene chloride and subjected to careful chromatography on Florisil.

Fraction 1 (30 ml., CH_2Cl_2),
needles, 20 mg., m. p., 204~206°C
Fraction 2 (30 ml., CH_2Cl_2),
needles, 50 mg., m. p., 208~210°C
Fraction 3 (30 ml., CH_2Cl_2),
needles, 35 mg., m. p., 210~240°C
Fraction 4 (30 ml., CH_2Cl_2),
needles, 35 mg., m. p., 295~300°C
Fraction 5 (50 ml., CH_2Cl_2)

Fraction 6 (50 ml., ether)

The substances from Fractions 1 and 2 were the same and did not show depression of m. p. on admixture. They were combined and recrystallized to give an analytical sample, m. p. 208~210°C.

Anal. Found: C, 73.77; H, 9.67. Calcd. for $C_{30}H_{48}O_5$: C, 73.73; H, 9.90%.

As already discussed it is 25D-5β-spirostan-1β, 2β,3β-triol 1,2-acetonide (XIII).

The substance from Fraction 4 proved to be identical with tokorogenin acetonide (IIa), and the substance from Fraction 3 is, therefore, very likely a mixture of IIa and XIII.

Summary

(1) The correlation of tokorogenin (I) with the known steroid, 25D-5β-spirostan (IX), was established: Tokorogenin formed an acetonide (IIa), which could be tosylated and then hydrolyzed to give tokorogenin monotosylate (IVb). On treatment with alkali, IVb was led to anhydrotokorogenin (Va), which on oxidation with Py.-CrO_3 and subsequent reduction with $CrCl_2$ gave rise to an α,β-unsaturated ketone (VII). Catalytic hydrogenation of VII followed by the Huang-Minlon reaction furnished 25D-5β-spirostan (IX).

(2) In order to determine the configuration of the hydroxyl group at C_3, IIa was oxidized with Py.-CrO_3 and the product was reduced with $LiAlH_4$ to give a mixture of IIa and XIII. Careful chromatography of the mixture revealed that the substance eluted first was XIII, while the second which eluted from the column was IIa, thus leaving only the structural possibility of 25D-5β-spirostan-1β,2β,3α-triol (I) to tokorogenin.

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